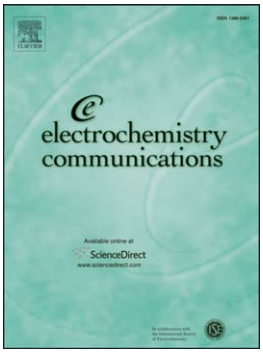

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Electrochemical detection of cytosine and 5-methylcytosine on Au(111) surfaces

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Abstract

In this communication we report a voltammetric study of the adsorption-desorption of cytosine (C) and methylcytosine (mC) on well-defined gold (Au) electrodes. The voltammetric measurements clearly indicate that these processes are extremely sensitive to the Au surface structure and in particular to the presence of (111) surface domains. Interestingly, on Au(111) surfaces, a linear correlation between the C and mC concentrations (logarithm scale) and the peak potential of the main voltammetric feature is found. In addition, in the simultaneous presence of both molecules, mC governs the electrochemical response, which has allowed its accurate quantification in C-mC mixtures. *In situ* FTIR spectroscopic measurements have been carried out to deepen on this mC electrochemical sensitivity. This research may contribute to the future development of an electrochemical sensor for the determination of the degree of methylation in DNA.

Keywords: Cytosine, 5-methylcytosine, Au(111), Sensor, Electrochemistry

1. Introduction

DNA methylation [1] is an epigenetic process that affects the regulation of gene expression thus playing an important role in serious human diseases such as tumours [2-4] and sterility [5, 6], among others. Consequently, the development of analytical procedures for its detection and quantification is a matter of outstanding significance. In this sense, and despite some methods are already available [7-11], most of them are time and DNA material consuming. Therefore, novel, fast, sensitive, simple, and economical methods for DNA methylation assays are still sought. Due to its high sensitivity, rapid response and ease of implementation, voltammetric techniques are very promising for DNA studies [12-15]. Thus, some approaches using glassy carbon [12, 16], boron doped diamond [14, 17], nanocarbon film [15, 18], screen printed graphite [13, 19] and graphene oxide [20, 21] based electrodes have been already reported for the electrochemical detection of free DNA bases, nucleosides, nucleotides and oligonucleotides under different experimental conditions.

On the other hand, it is also known that DNA bases (nucleobases) can be adsorbed on some metal solid surfaces, particularly, on Au electrodes [22-30]**.** This adsorption-desorption process has been also shown to be sensitive to the surface structure of the Au electrode, that is, to its specific surface atomic arrangement. Taking into account all these aspects, this contribution aims to evaluate the adsorption-desorption properties of cytosine (C) and methylcytosine (mC) on well-defined Au surfaces by cyclic voltammetry and infrared spectroscopy.

2. Experimental

Gold single crystal electrodes were prepared from small (2–3 mm diameter) crystal beads as reported previously [31]. Larger electrodes (4-5 mm in diameter) were used for the spectroelectrochemical experiments. Prior to each electrochemical experiment, the single crystal electrodes were flame-annealed and quenched with ultrapure water (Milli-Q 18.2 MΩ cm). A gold bead fully immersed in the electrochemical cell solution was

also used as a polyoriented gold electrode. The quality of the gold electrodes was verified by cyclic voltammetry in 0.1 M phosphate buffer solution (pH=7) [32]. The phosphate buffer solution was prepared using a certain ratio of NaH_2PO_4 and Na_2HPO_4 (Panreac 99% purity). The pH of the solution was checked with a Crison 507 pH-meter. Cytosine (C) and 5-methylcytosine (mC) were obtained at the highest analytical grade available (Sigma Aldrich) and were used as received. C and mC solutions were prepared in 0.1 M phosphate buffer pH 7 solution.

Voltammetric experiments were carried out in a standard three-electrode electrochemical cell. The electrode potential was controlled by a PGSTAT302N (Metrohm Autolab) system. A gold wire was used as counter electrode. The potentials were measured against a reversible hydrogen electrode (RHE) connected to the cell through a Luggin capillary. Solutions were deaerated with Ar (99.999%, AlphaGaz). All experiments were performed at room temperature (22 ± 2 ºC). As usual, Au single crystal electrodes contacted the solution through the hanging meniscus configuration.

In situ FTIR spectra were acquired with a Nicolet Magna 850 spectrometer equipped with a liquid nitrogen cooled MCT detector. The spectroelectrochemical cell was provided with a prismatic CaF_2 window bevelled at 60º. The optical path through the solution was minimised by pressing the electrode against the window as described previously [33]. Spectra shown are composed of 100 interferograms collected with a resolution of 8 cm^{-1} and p polarized light. Spectra are presented as absorbance, according to $A= - \log (R/R_0)$ where R and R_0 are the reflectance corresponding to the single-beam spectra obtained at the sample and reference potentials, respectively. All the spectroelectrochemical experiments were performed in 1 mM cytosine or 1 mM methylcytosine in phosphate buffer (pH 7) at room temperature. Potential control during the spectroelectrochemical experiments was maintained using an EG&G PARC 175 signal generator in combination with an eDAQ EA161 potentiostat, with a RHE and a gold wire as reference and counter electrodes, respectively. FTIR experiments were performed as follows: the electrode was immersed at controlled potential (1.3 V) in the working solution and the potential was kept until the electrode was pressed against the CaF_2 window. Then, at that potential, a spectrum was recorded and subsequently the potential was moved to the next one where a new spectrum was recorded. In this way, different spectra were recorded. The spectrum collected at 0.1 V was taken as the reference one.

3. Results and Discussion

Figure 1 shows the voltammetric profiles obtained with a Au bead (polyoriented surface) and with the three basal plane electrodes (Au(100), Au(110) and Au(111)) in 0.1 M phosphate buffer solution (pH=7) in presence of C (figure 1A) and mC (figure 1B) both at 100 µM. Results obtained clearly indicate how sensitive is the adsorption desorption of both molecules to the surface structure of the Au electrodes. Thus, distinct and characteristic voltammetric signals are recorded for each surface orientation. These features are obviously different to those obtained in absence of C or mC which are known to be related to the phosphate anion adsorption-desorption on Au surfaces (Inset figure 1B) [32]. Noticeably, the voltammetric profiles of the polyoriented surface show the presence of multiple and well-defined contributions which can be easily assigned with those coming from the Au single crystals.

Among the different voltammetric features, the sharp peak at about 0.75 V and 0.7 V for C and mC, respectively, obtained with the Au(111) electrode in the negative-sweep is particularly relevant. This contribution (also observed with the polyoriented surface) is clearly more intense for mC than for C for equivalent concentrations. Interestingly, when C or mC concentrations are systematically varied, a clear dependence between the potential of this peak and the concentration is observed. This dependence is depicted in figure 2A for mC where, for sake of comparison, the voltammetric response of a Au(111) in absence of mC is also included (dotted line). Such results exhibit that the position of the peak varies linearly with the concentration of mC (*logarithm* scale) shifting to more negative potentials and becoming more intense for increasing mC concentrations. A similar tendency is also observed for C (results not shown). However, it is worth noting that not only the peak intensity is different between mC and C but also the potential range where the peaks appear and the distinct slopes of the plot Ep vs concentration, being higher, in absolute value, for mC (0.063 V dec^{-1}) than for C (0.0388 V dec^{-1}).

Subsequently, additional experiments were performed in which the concentration of mC was systematically varied in presence of a constant and high concentration of C (200 µM). Results obtained are shown in figure 2B and they demonstrate

that an accurate determination of the mC concentration can be performed by simple assessment of the peak potential under study. In fact, by plotting the corresponding E vs [mC], it is possible to prove that a 10 μM addition of mC into the buffer solution effectively shifts the peak potential thereby allowing its determination. These results indicate that, in C-mC mixtures, where a competitive adsorption between these two molecules is expected to take place, mC is the key molecule determining the resulting electrochemical signal. To verify this finding, an experiment was performed keeping a constant and high concentration of mC (200 μM) and systematically changing the concentration of C. Findings obtained showed that the addition of C (between 0-200 μM) does not significantly perturb the peak potential position which remains essentially constant (0.69 ± 0.01 V). These results point out that the concentration of mC determines the potential position of the peak. Obviously, this does not mean that C is not co-adsorbed. In fact, the peak potential obtained for the same [mC] is different when C is present, thus suggesting a co-adsorption as previously reported with other nucleobases [34, 35]. This fact will difficult a direct electroanalytical determination of mC in presence of C because some pre-analysis, including a pre-determination of C as well as some previous calibration protocols will be required. In any case, these evidences probe that the potential position of the peak under study is mainly controlled by the [mC].

To deepen in this mC electrochemical sensitivity, spectroelectrochemical experiments were subsequently performed. However, preliminary voltammetric experiments were carried out to electrochemically evidence at which potentials C and mC are adsorbed or desorbed. To do that, two electrochemical cells were used; one of them containing a 200 μM of either C or mC in phosphate buffer solution and a second one just containing phosphate buffer solution. The Au(111) electrode was initially cycled in the cell one containing C or mC and then the scan was stopped at the upper or at the lower limit potential. Irrespectively of the selected potential, the electrode was taken out the first cell and without being rinsed, it was immersed into the second cell under the same potential at which was taken out from the first one. Such simple electrochemical experiments demonstrated that if the electrode is taken out at 1.3 V, the voltammetric profile during the first negative going sweep resembles that observed in the presence of

C or mC, while in the subsequent positive scan, the voltammetric profile shows the characteristic profile of an Au(111) in phosphate buffer solution. On the other hand, when the electrode is taken out at the lower potential limit, the subsequent voltammetric profile does not show any of the C or mC characteristic voltammetric features. These experiments clearly indicate that both C and mC are desorbed during the negative potential sweep and adsorbed during the positive one. This behavior is in good agreement with previous findings by Wandlowski et al. who claimed that cytosine formed a 'dilute' disordered phase on Au(111) at lower potentials [25], and also with Ataka and Osawa who using *in situ* infrared measurements reported that on evaporated thin Au film electrodes (formed by small particles with preferentially (111) orientation), cytosine is only weakly physisorbed at negative potentials and chemisorbed at positive ones [36].

Keeping in mind this situation, figure 3 shows the FTIR spectra for C and mC during the positive and negative sweeps (from 1.3 to 0.4 V and *vice versa*). For sensitivity reasons, during the IR experiments, C or mC concentrations were increased up to 1 mM. In addition, to avoid the interference of the –OH bending mode of H_2O at ca. 1650 cm^{-1}, spectra were obtained in D_2O solutions where the –O-D bending band is shifted to about 1200 cm^{-1}. Finally, and due to the fact that the reference spectra were collected at 0.1 V (where C and mC are not chemisorbed but physisorbed), positive bands are due to species being adsorbed on the surface at the sample potential, while negative bands correspond to the desorption of species adsorbed at the reference potential. At first sight, both collections of spectra show similar spectroscopic features as well as the same quasi-reversible spectra evolution with the electrode potential. In particular, for increasing potential values, positive bands at 1565, 1538 and 1285 cm^{-1} are observed for both chemisorbed C and mC. Additionally, a band at 1632 cm^{-1} is found for C which shifts to higher wavenumbers (1648 cm^{-1}) for mC. According to previous findings [36], the latter band is attributed to the C=O stretching of the chemisorbed molecule whereas the others can be associated with the metal coordinated to the N3 position of the molecule, to the N3-C4, N1-C2 and to N1-C6, C5-C6 stretching, respectively. Interestingly, also negative bands are observed for increasing potential values reflecting desorption of the species physisorbed at the reference potential (0.1 V). Negative bands at 1605, 1585 and

1508 cm^{-1} are observed for both C and mC. The bands at 1605, 1585 cm^{-1} are related to C4-C5, C4-NH_2 and δNH_2 contributions while the signal at 1508 cm^{-1} is due to N1-C2, N1-C6 and C2-N3 contributions. In addition, the contribution of the C=O stretching of physisorbed mC is again shifted to higher wavenumbers (1662 cm^{-1}) in comparison with C (1646 cm^{-1}). Finally, a band at 1388 cm^{-1} attributed to the N1-C6, C5-C6 contributions is observed with C but not for mC, which could be due to the C5 methylation. Nevertheless, due to the similarities of the spectra, the results obtained do not allow shedding light into the preferential adsorption of mC against C Au(111) surfaces. More studies are in progress to understand these findings.

4. Conclusions

In this work, a voltammetric study of the adsorption of cytosine (C) and methylcytosine (mC) on well-defined gold (Au) electrodes is reported. The voltammetric measurements clearly indicate, for the first time, that the adsorption of C and mC in 0.1 M phosphate buffer solution (pH=7) is extremely sensitive to the Au surface structure and more specifically to the (111) surface domains. Interestingly, with Au(111) surfaces, the electrochemical response has been found to be dependent on the C and mC concentrations. However, mC seems to govern the electrochemical response, which has been used for its precise quantification in the presence of C. Spectroelectrochemical measurements provided no clear evidence for understanding the competitive adsorption of mC against C. More work is in progress to elucidate the preferential and competitive adsorption of mC on Au(111) surfaces.

Acknowledgements

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References

[1] B. Alberts, Molecular Biology of the Cell: Reference Edition, Taylor & Francis, 2008.

[2] S.A. Belinsky, K.J. Nikula, W.A. Palmisano, R. Michels, G. Saccomanno, E. Gabrielson, S.B. Baylin, J.G. Herman, Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis, P. Natl. Acad. Sci. USA 95 (1998) 11891-11896.

[3] K.V. Donkena, C.Y.F. Young, D.J. Tindall, Oxidative stress and DNA methylation in prostate cancer, Obstet. Gynecol. Int. 2010 (2010) 302051.

[4] S.B. Baylin, J.G. Herman, J.R. Graff, P.M. Vertino, J.P. Issa, Alterations in DNA methylation: A fundamental aspect of neoplasia, Adv. Cancer Res. 72 (1998) 141-196.

[5] P. Navarro-Costa, P. Nogueira, M. Carvalho, F. Leal, I. Cordeiro, C. Calhaz-Jorge, J. Goncalves, C.E. Plancha, Incorrect DNA methylation of the DAZL promoter CpG island associates with defective human sperm(dagger), Hum. Reprod. 25 (2010) 2647-2654.

[6] W. Wu, O. Shen, Y. Qin, X. Niu, C. Lu, Y. Xia, L. Song, S. Wang, X. Wang, Idiopathic Male Infertility Is Strongly Associated with Aberrant Promoter Methylation of Methylenetetrahydrofolate Reductase (MTHFR), Plos One 5 (2010) e13884.

[7] M. Schwarz, G. Wiesbeck, J. Hummel, W. Kunz, Effect of ethanol on dimethylnitrosamine activation and DNA synthesis in rat liver, Carcinogenesis 3 (1982) 1071-1075.

[8] J.A. Swenberg, M.C. Dyroff, M.A. Bedell, O4-ethyldeoxythymidine, but not O6-ethyldeoxyguanosine, accumulates in hepatocyte DNA of rats exposed continuously to diethylnitrosamine, P. Natl. Acad. Sci. USA 81 (1984) 1692-1695.

[9] R.C. Gupta, M.V. Reddy, K. Randerath, 32P-postlabeling analysis of non-radioactive aromatic carcinogen - DNA adducts, Carcinogenesis 3 (1982) 1081-1092.

[10] P.G. Foiles, S.A. Akerkar, S.G. Carmella, M. Kagan, G.D. Stoner, J.H. Resau, S.S. Hecht, Mass spectrometric analysis of tobacco-specific nitrosamine - DNA adducts in smokers and nonsmokers, Chem. Res. Toxicol. 4 (1991) 364-368.

[11] R. Singh, P.B. Farmer, Liquid chromatography-electrospray ionization-mass spectrometry: The future of DNA adduct detection, Carcinogenesis 27 (2006) 178-196.

[12] A.M. Oliveira-Brett, J.A.P. Piedade, L.A. Silva, V.C. Diculescu, Voltammetric determination of all DNA nucleotides, Anal. Biochem. 332 (2004) 321-329.

[13] A. Brotons, L.A. Mas, J.P. Metters, C.E. Banks, J. Iniesta, Voltammetric behaviour of free DNA bases, methylcytosine and oligonucleotides at disposable screen printed graphite electrode platforms, Analyst 138 (2013) 5239-5249.

[14] T.A. Ivandini, K. Honda, T.N. Rao, A. Fujishima, Y. Einaga, Simultaneous detection of purine and pyrimidine at highly boron-doped diamond electrodes by using liquid chromatography, Talanta 71 (2007) 648-655.

[15] D. Kato, N. Sekioka, A. Ueda, R. Kurita, S. Hirono, K. Suzuki, O. Niwa, A nanocarbon film electrode as a platform for exploring DNA methylation, J. Am. Chem. Soc. 130 (2008) 3716-3717.

[16] A.M. Oliveira-Brett, V. Diculescu, J.A.P. Piedade, Electrochemical oxidation mechanism of guanine and adenine using a glassy carbon microelectrode, Bioelectrochemistry 55 (2002) 61-62.

[17] T.A. Ivandini, B.V. Sarada, T.N. Rao, A. Fujishima, Electrochemical oxidation of underivatized-nucleic acids at highly boron-doped diamond electrodes, Analyst 128 (2003) 924-929.

[18] D. Kato, K. Goto, S.-i. Fujii, A. Takatsu, S. Hirono, O. Niwa, Electrochemical DNA Methylation Detection for Enzymatically Digested CpG Oligonucleotides, Anal. Chem. 83 (2011) 7595-7599.

[19] A. Brotons, I. Sanjuan, C.E. Banks, F.J. Vidal-Iglesias, J. Solla-Gullón, J. Iniesta, Voltammetric Behaviour of 7-Methylguanine Using Screen-printed Graphite Electrodes: towards a Guanine Methylation Electrochemical Sensor, Electroanal. 27 (2015) 2766-2772.

[20] M. Zhou, Y. Zhai, S. Dong, Electrochemical Sensing and Biosensing Platform Based on Chemically Reduced Graphene Oxide, Anal. Chem. 81 (2009) 5603-5613.

[21] X. Zheng, L. Wang, Direct Electrocatalytic Oxidation and Simultaneous Determination of 5-Methylcytosine and Cytosine at Electrochemically Reduced Graphene Modified Glassy Carbon Electrode, Electroanal. 25 (2013) 1697-1705.

[22] M. Rueda, F. Prieto, A. Rodes, J.M. Delgado, In situ infrared study of adenine adsorption on gold electrodes in acid media, Electrochim. Acta 82 (2012) 534-542.

[23] A. Rodes, M. Rueda, F. Prieto, C. Prado, J.M. Feliu, A. Aldaz, Adenine Adsorption at Single Crystal and Thin-Film Gold Electrodes: An In Situ Infrared Spectroscopy Study, J. Phys. Chem. C 113 (2009) 18784-18794.

[24] C. Prado, F. Prieto, M. Rueda, J. Feliu, A. Aldaz, Adenine adsorption on Au(1 1 1) and Au(1 0 0) electrodes: Characterisation, surface reconstruction effects and thermodynamic study, Electrochim. Acta 52 (2007) 3168-3180.

[25] T. Wandlowski, D. Lampner, S.M. Lindsay, Structure and stability of cytosine adlayers on Au(111): an in-situ STM study, J. Electroanal. Chem. 404 (1996) 215-226.

[26] B. Roelfs, E. Bunge, C. Schröter, T. Solomun, H. Meyer, R.J. Nichols, H. Baumgärtel, Adsorption of Thymine on Gold Single-Crystal Electrodes, J. Phys. Chem. B 101 (1997) 754-765.

[27] T. Wandlowski, M.H. Hölzle, Structural and Thermodynamic Aspects of Phase Transitions in Uracil Adlayers. A Chronocoulometric Study, Langmuir 12 (1996) 6604-6615.

[28] N.J. Tao, J.A. DeRose, S.M. Lindsay, Self-assembly of molecular superstructures studied by in situ scanning tunneling microscopy: DNA bases on Au(111), J. Phys. Chem. 97 (1993) 910-919.

[29] T. Boland, B.D. Ratner, Two-Dimensional Assembly of Purines and Pyrimidines on Au(111), Langmuir 10 (1994) 3845-3852.

[30] L. Fojt, V. Vetterl, T. Doneux, Adsorption and two-dimensional condensation of 5-methylcytosine, Bioelectrochemistry 75 (2009) 89-94.

[31] J. Clavilier, D. Armand, S.G. Sun, M. Petit, Electrochemical adsorption behaviour of platinum stepped surfaces in sulphuric acid solutions, J. Electroanal. Chem. 205 (1986) 267-277.

[32] A. Martins, A. Queirós, F. Silva, Surface-Structure-Sensitive Adsorption of Adenine on Gold Electrodes, ChemPhysChem 6 (2005) 1056-1060.

[33] T. Iwasita and F.C. Nart, In-Situ Fourier Transform Infrared Spectroscopy: A Tool to Characterize the Metal-Electrolyte Interface at a Molecular Level, in Advances in Electrochemical Science and Engineering, Volume 4 (eds H. Gerischer and C.W. Tobias), Wiley-VCH Verlag GmbH, Weinheim, Germany, 1995, pp.123-216. DOI: 10.1002/9783527616787.ch3.

[34] A.P.M. Camargo, H. Baumgartel, C. Donner, Coadsorption of the DNA bases thymine and adenine at the Au(111) electrode, Physical Chemistry Chemical Physics 5 (2003) 1657-1664.

[35] A. P. M. Camargo, H. Baumgartel, C. Donner, Adsorption behaviour of DNA bases at the Au(111) electrode, PhysChemComm 5 (2002) 151-157.

[36] K. Ataka, M. Osawa, In situ infrared study of cytosine adsorption on gold electrodes, J. Electroanal. Chem. 460 (1999) 188-196.

Figure captions

Figure 1. Voltammetric response for cytosine (A) and 5-methylcytosine (B) adsorption-desorption ([C]=[mC]=100 µM) on polyoriented gold, Au(111), Au(110) and Au(100) single crystals in 0.1 M phosphate buffer pH=7 solution. Inset displays the blank voltammetric profiles. Scan rate: 50 mV s^{-1}. For sake of comparison, the response of the polyoriented Au surface is multiplied by a factor of 3.

Figure 2. Voltammetric response of a Au(111) in a 0.1 M phosphate buffer pH=7 solution with increasing amounts of mC in absence (A) and presence of 200 µM of C (B). Dotted line in (A) corresponds to the blank voltammogram. In both cases, a zoomed view of the spike and a peak potential vs [mC] (*log* scale) plot are shown. Scan rate: 50 mV s^{-1}.

Figure 3. Spectra obtained at different potentials, as indicated, on Au (111) electrode in phosphate buffer pH=7 solution prepared in D_2O containing 1 mM C or mC. Each spectrum is composed of 100 interferograms with a resolution of 8 cm^{-1} and p-polarized light. Reference spectra were obtained at 0.1 V.

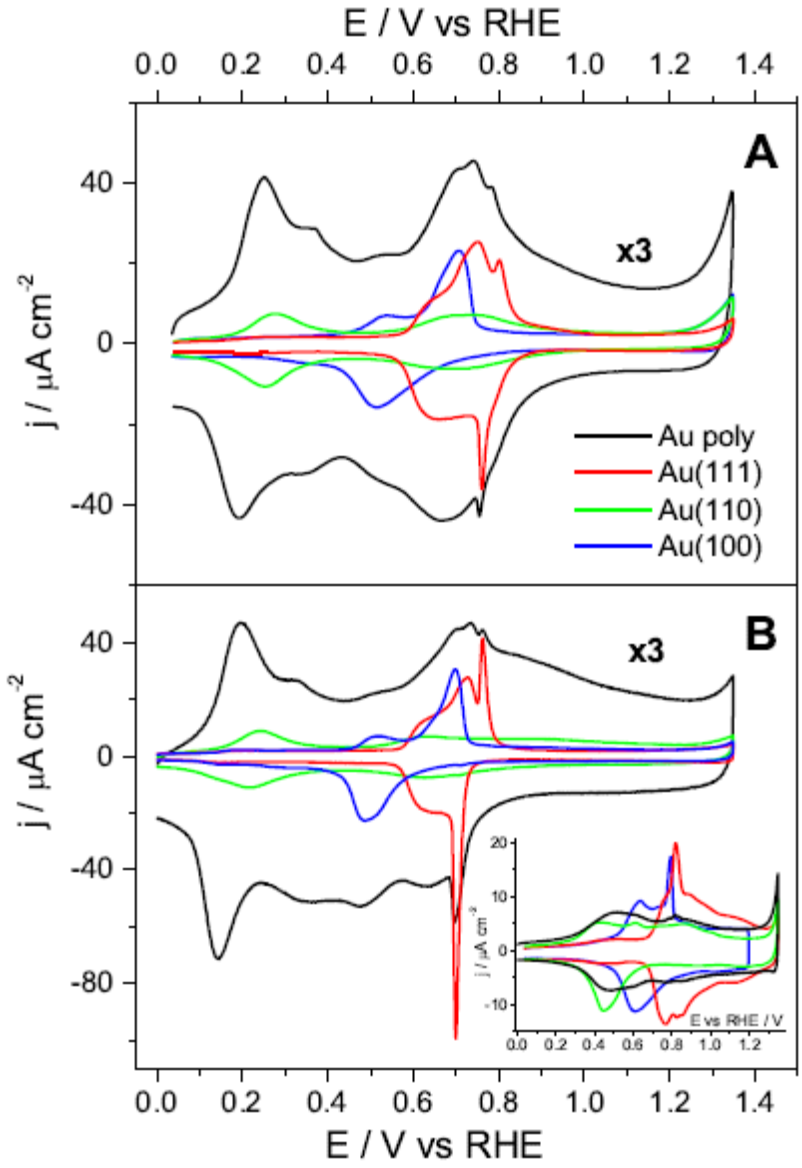


Figure 1

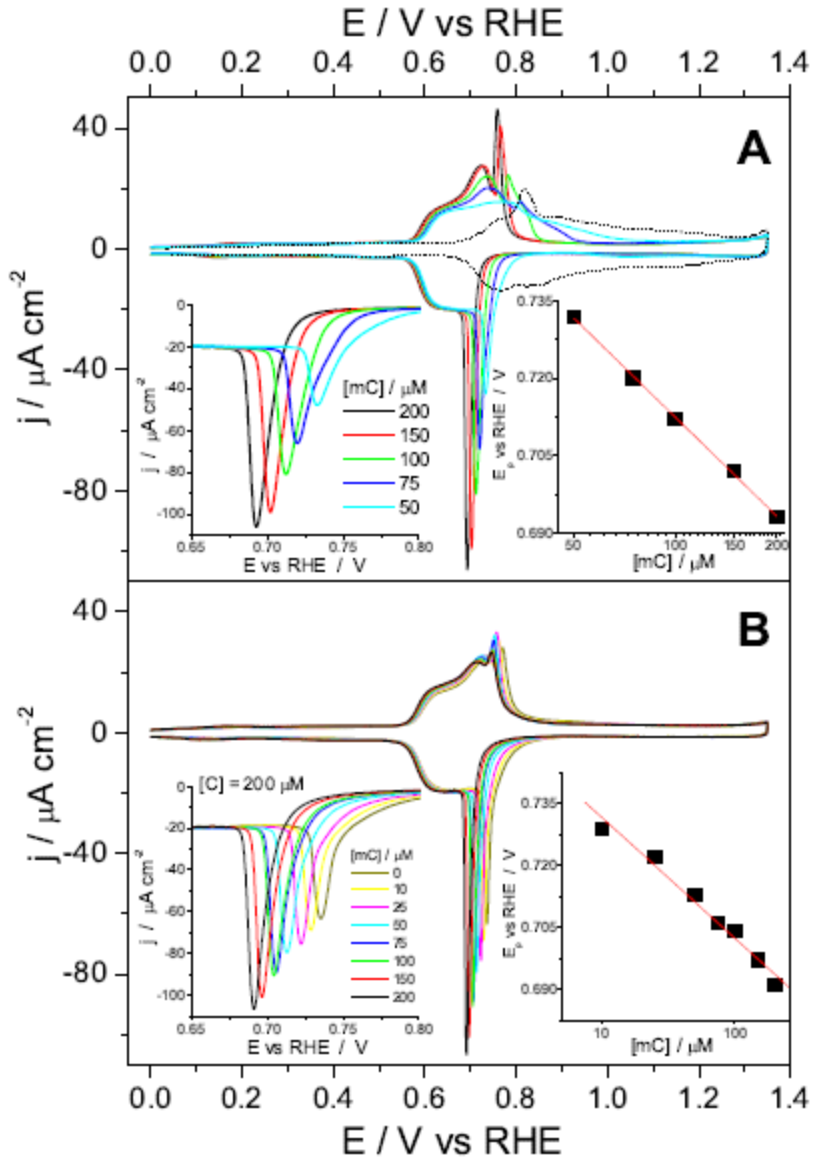


Figure 2

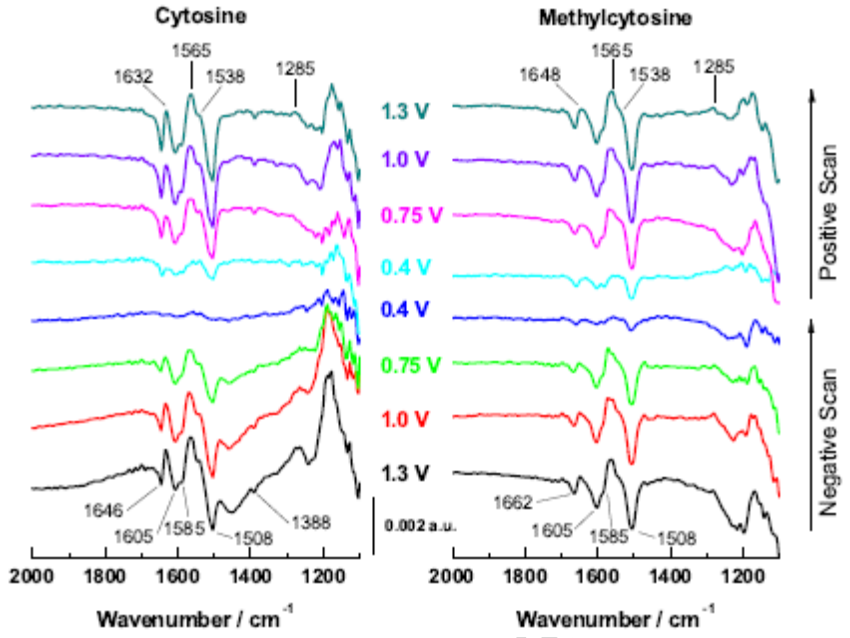
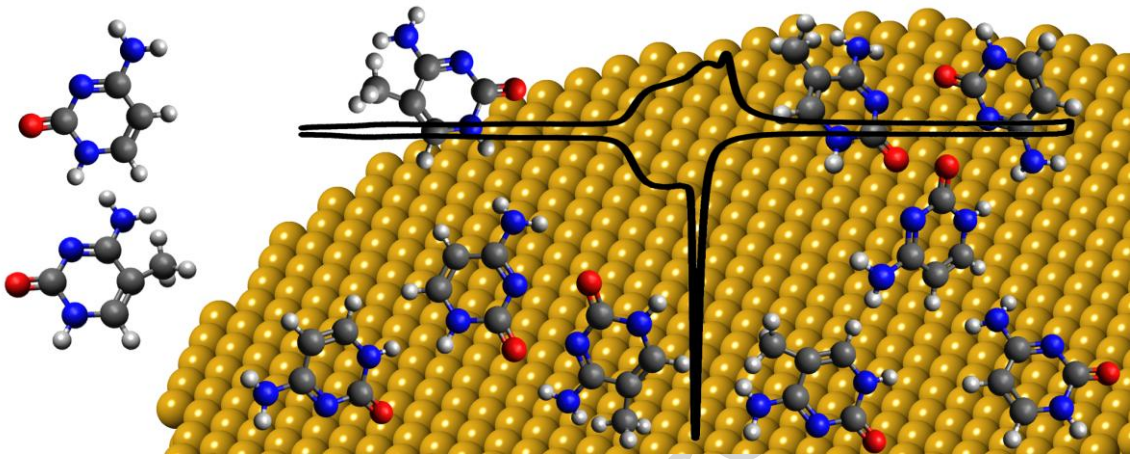


Figure 3



Graphical abstract

Highlights

"Electrochemical detection of cytosine and 5-methylcytosine on Au(111) surfaces" by Ariadna Brotons, Rosa M. Arán-Ais, Juan M. Feliu, Vicente Montiel, Jesús Iniesta, Francisco J. Vidal-Iglesias and José Solla-Gullón

- Adsorption-desorption of both molecules on Au is surface structure sensitive.
- On Au(111), a linear correlation between concentration and peak potential is observed.
- The presence of mC mainly determines the resulting electrochemical response.
- In mC-C mixtures, the presence of mC can be electrochemically determined.
- FTIRs studies do not provide clear evidences of the mC voltammetric selectivity.